A. Claims 1, 19, 22-25 and 33-37

Independent claim 1 is drawn to a process for fragmenting and labeling DNA and chimeric DNA-RNA polymers that includes chemically fragmenting the polymer to produce a plurality of fragments and attaching a labeling agent on the fragments. The cited claims of the '179 patent do not teach or suggest such a process.

The cited '179 claims limit the claimed subject matter to RNA only. In particular, independent claims 1, 22, 26, and 30 all recite in part: "[A] process for labeling a synthetic or natural ribonucleic acid (RNA), comprising: fragmenting the RNA...." Independent claim 31 recites: "[A] process for labeling a natural ribonucleic acid (RNA), comprising: fragmenting the natural RNA...," and independent claim 33 recites "[A] process for labeling a synthetic or natural ribonucleic acid (RNA), comprising: non-specifically fragmenting the RNA...." None of the '179 claims include a feature drawn to DNA or chimeric DNA-RNA. As such, the '179 claims do not teach or suggest instant claim 1 and claims 19, 22-25 and 33-37 dependent thereon.

In addition, instant claims 1, 19, 22-25 and 33-37 would not have been rendered obvious in view of the cited '179 claims. As detailed above, the '179 patent claims are limited to RNA. The instant claims' DNA and DNA-RNA polymer feature is not an obvious variation of RNA. It is well known and established in the art that the chemical characteristics and behavior of DNA and RNA differ significantly. As detailed in the remarks below responding to the §103 rejections, compounds found to effectively fragment RNA do not work on DNA.

Thus, for at least these reasons, claims 1, 19, 22-25 and 33-37 are not obviousness-type double patenting of the '179 patent claims. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

B. Claims 26-32

Independent claim 26 is drawn to a process for fragmenting and labeling DNA or RNA nucleic acid that includes chemically fragmenting the nucleic acid in the presence of specific multivalent metal cation, i.e., Ba^{2+}, Be^{2+}, Cd^{2+}, Ce^{3+}, Cr^{3+}, Eu^{3+}, Fe^{2+}, In^{3+}, Lu^{3+}, Ni^{2+}, Pb^{2+}, Ru^{3+}, Sr^{2+}, Tb^{3+}, Tm^{3+} and Yb^{3+}. The cited claims of the '179 patent do not teach or suggest such a process.

The cited '179 claims recite metal cations in general (claim 7), or are limited to specific metal cations Mg^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+} and Zn^{2+} combined with a chemical catalyst (claim 17). As such, the '179 claims do not teach or suggest the method of instant claim 26 and claims 27-32 dependent thereon. The broad recitation of metal cations in claim 7 fails to provide any teaching or suggestion that would have pointed one of ordinary skill in the art to select the specific multivalent metal cations featured in instant claim 26.

As disclosed in the specification, Applicants found that the labeling of DNA and RNA is metal cation sensitive. (See specification, Example 6 and Table 1, Example 7 and Table 2, and Example 3 and Table 3). Moreover, Applicants found that specific cations recited in claim 17, i.e., Zn^{2+} and Mn^{2+}, exhibited relatively low activity. (See, specification at page 24, lines 27-29).

Thus, for at least these reasons, claims 26-32 are not obviousness-type double patenting of the '179 patent claims. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

II. <u>Rejection Under Section 112</u>

Claims 1-37 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite. The Office Action states that claims 1-37 are indefinite because it is unclear how the language a "substantially aqueous solution differs from an "aqueous solution."

Amended independent claims 1, 4 and 26 feature only an "aqueous solution" and no longer recite a "substantially aqueous solution." However, it is respectfully submitted that the

phrase "aqueous solution" would be understood in the art to encompass solutions that are not completely aqueous. Any further recitation of "said aqueous solution" in any of the dependent claims clearly refers to such an aqueous solution. Thus, claims 1-37 clearly define the claimed subject matter. Accordingly, reconsideration and withdrawal of this aspect of the rejection are respectfully requested.

The Office Action states that claim 3 is indefinite because the term "reagents" lacks antecedent basis. Amended claim 3 no longer contains this term. Accordingly, Applicants respectfully request reconsideration and withdrawal of this aspect of the rejection.

The Office Action alleges that claims 15 and 31 are vague and indefinite because chemical compounds are recited in the abbreviated form. The Examiner suggests that the complete name for these compounds be used.

In response, Applicants first note that claims 15 and 25 contain the abbreviations. Thus, as suggested by the Examiner, Applicants have amended claims 15 and 25 to write out the full chemical names. Accordingly, this aspect of the rejection is overcome and should be withdrawn. Reconsideration and withdrawal of this aspect of the rejection are respectfully requested.

III. Rejections Under Section 103

A. Claims 1, 4-6, 8, 10-13, 19-20, 23-26, 29 and 32-37 Over Morrow and Mirzabekov

The Office Action rejects claims 1, 4-6, 8, 10-13, 19-20, 23-26, 29 and 32-37 under 35 U.S.C. §103(a) over U.S. Patent No. 5,684,149 to Morrow ("Morrow") in view of U.S. Patent No. 5,981,734 to Mirzabekow et al. ("Mirzabekow"). Applicants respectfully traverse this rejection.

Independent claims 1, 4 and 26 are drawn toward a process for fragmenting DNA or RNA in the presence of multivalent metal cation to produce a plurality of DNA or RNA fragments having freed terminal phosphates for further reaction, and labeling the fragments by attaching a labeling agent at freed terminal phosphates located at the 3' end and/or 5' end

of the fragments. Morrow and Mirzabekow, either alone or in combination, fail to teach or suggest such a process.

The Office Action cites Morrow for teaching metal complexes that promote the cleavage of RNA. The Office Action acknowledges that Morrow does not disclose a method to fragment DNA but contends that DNA and RNA have the same nucleotide molecular (sic), and thus, one of ordinary skill in the art would have recognized that Morrow's method to fragment RNA would also work with DNA. Applicants respectfully disagree with this position.

Morrow describes metal complexes with catalytic behavior in RNA cleavage by promoting transesterification of the phosphate diester linkage of RNA (col. 1, lines 9-12 and col. 3, lines 61-63). The entire disclosure of Morrow focuses exclusively on RNA cleavage. Morrow does not provide any suggestion that the described metal complexes would actively cleave DNA. Completely opposite to the position stated in the Office Action, one of ordinary skill in the art would have recognized that the metal complexes would be specific for RNA and would not have considered any possible catalytic activity in DNA or DNA/RNA molecules.

Morrow teaches that nucleases that cleave RNA by promoting transesterification are desired because they are not only effective, but in particular, they are selective for RNA (col. 13, lines 56-60). Examples III and IV in Morrow illustrate the catalytic effectiveness of various embodiments on the cleavage of tRNA.

Morrow further discloses that RNA structure has a dramatic effect on the rate of cleavage by the metal complexes and that RNA with a large degree of secondary and tertiary structure are not readily cleaved (col. 14, lines 1-10). Moreover, transesterification catalysts are unable to cleave RNA in a DNA-RNA hybrid. Morrow speculates that the conformation of RNA in a double-helical form protects it from cleavage (col. 14, lines 10-16).

In Example V, Morrow provides further evidence for nuclease activity that is specific for single stranded RNA. In particular, a 20-base oligonucleotide annealed to a t-RNA protected all sites on the t-RNA sequence complementary to the DNA hybrid (col. 14, lines 30-33). Morrow speculates that the more rigid conformation of RNA in duplex form plays a large role in the observed loss of nuclease activity. Morrow further teaches that phosphate esters of RNA in a helical form are in the wrong conformation to undergo nucleophilic attack and displacement of the 5' hydroxyl (col. 15, lines 9-12)

Morrow teaches away from using metal cations to cleave DNA and chimeric DNA-RNA polymers as claimed. The catalytic effectiveness of the metal complexes in Morrow is limited to single-stranded RNA. Morrow fails to provide any teaching that would have suggested or motivated one of ordinary skill in the art to apply its teachings to a process for fragmenting DNA and chimeric DNA-RNA molecules as claimed.

Morrow also does not teach or suggest labeling DNA or RNA as claimed. The only location in the reference at which Morrow refers to labeling RNA is in the description of Figure 3(b) at column 12, lines 62-65. Here, Morrow describes the detection of t-RNA fragmentation by ^{32}P labeling and sequence gel electrophoresis. Morrow discloses only that the t-RNA fragments are labeled at the 3' end without providing any further specifics. One of ordinary skill in the art would have presumed a kinase reaction ^{32}P labeling at the 3' OH group. In no way does the disclosure of such a label anticipate the specific labeling as claimed. Claims 1, 4 and 26 recite "attaching a labeling agent on a plurality of said fragments at freed terminal phosphates located at the 3' end and/or 5' end of said fragments." Morrow does not teach or suggest such a feature.

The secondary reference, Mirzabekow, does not rectify the failed teachings of Morrow. Mirzabekov discloses labeling nucleic acids at their terminal ends. The label is, however, not located at the terminal phosphate, as required by claims 1, 4-6, 8, 10-13, 19-20, 23-26, 29 and 32-37 but rather is located (i) for DNA, on the sugar moiety after the base has

been eliminated during depurination in an acidic medium (see Figure 1) or (ii) for RNA, at the 2' or 3' position of the sugar moiety after the ribose cycle is opened during oxidation (see column 5, lines 33-38 and lines 40-43, and Figure 2). Therefore, Mirzabekov fails to teach labeling a DNA or RNA fragment at a freed terminal phosphate. Mirzabekov also fails to teach or suggest any fragmentation technique whatsoever.

For at least these reasons, Morrow and Mirzabekow, alone or in combination, do not teach or suggest, and would not have rendered obvious, all of the features as claimed. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1, 4 and 26, and claims 5-6, 8, 10-13, 19-20, 23-25, 29 and 32-37 dependent thereon.

B. Claims 2, 21-22, 27-28 and 30-31 Over Morrow, Mirzabekov et al. and Szostak

Claims 2, 21-22, 27-28 and 30-31 are rejected under 35 U.S.C. §103(a) over Morrow, in view of Mirzabekow, and further in view of U.S. Patent No. 5,688,670 to Szostak et al. ("Szostak"). Applicants respectfully traverse this rejection.

As detailed in the above paragraphs, the combination of Morrow and Mirzabekow does not teach or suggest the claimed fragmenting and labeling process. In addition, Szostak does not resolve their deficiencies.

The Office Action cites Szostak for its teaching of an RNA fragment comprised of at least one labeled thiophosphate nucleotide. Neither Morrow, Mirzabekow nor Szostak provide motivation to combine the references. Morrow is directed to a process of cleaving RNA. See Morrow at Abstract. Mirzabekow is directed to a method for labeling and immobilizing oligonucleotide molecules. See Mirzabekow at Abstract. Neither reference contains any teaching or suggestion pertaining to thiophosphate nucleotides.

Szostak, on the other hand, is directed to a method of isolating RNA that is capable of binding a ligand, and acting as a catalyst in a reaction involving the ligand. See Szostak at Abstract. Thiophosphates are employed in Szostak solely as a means for isolating catalytic

RNA. Neither reference provides any indication that thiophosphate nucleotides would have any value in the processes of Morrow or Mirzabekow.

Furthermore, even if thiophosphates were incorporated into DNA or RNA as allegedly taught by Szostak, the claimed methods would not have been achieved. As detailed above, the combination of Morrow and Mirzabekow would not have rendered the methods of claims 1, 4 and 16 obvious. Thus, claims 2, 21-22, 27-28 and 30-31, all dependent thereon, also would not have been obvious.

Szostak does not remedy the deficiencies of Morrow and Mirzabekow as detailed above. Szostak does not teach or suggest chemically fragmenting DNA, RNA, or chimeric DNA-RNA in the presence of multivalent metal cation, nor does it teach or suggest attaching a labeling agent at freed terminal phosphates located at the 3' end and/or 5' end.

Accordingly, the combination of Morrow, Mirzabekow and Szostak does not disclose, teach or suggest all of the limitations of the claimed invention. Reconsideration and withdrawal of the rejection are respectfully requested.

C. Claim 7 Over Morrow, Mirzabekov and Marliere

Claim 7 is rejected under 35 U.S.C. §103(a) over Morrow, in view of Mirzabekow, and further in view of U.S. Patent No. 5,407,797 to Marliere et al. ("Marliere"). Applicants respectfully traverse this rejection.

The Office Action cites Marliere for teaching the removal of unreacted molecules or fragments of molecules of probe and concludes that it would have been obvious to one of ordinary skill in the art to apply this step to the combined fragmenting and labeling method of Morrow and Mirzabekow.

Marliere does not rectify the deficiencies of Morrow and Mirzabekow as detailed above. Marliere does not teach or suggest chemically fragmenting DNA or RNA in the presence of at least one multivalent metal cation nor does it teach or suggest attaching a labeling agent at freed terminal phosphates located at the 3' end and/or 5' end. The

combination of Morrow, Mirzabekov and Marliere does not disclose, teach or suggest all of the limitations of the claimed invention.

Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

D. Claims 14-15 Over Morrow, Mirzabekov and Stefeno

Claims 14-15 are rejected under 35 U.S.C. §103(a) over Morrow, in view of Mirzabekov, and further in view of U.S. Patent No. 6,297,010 to Stefano ("Stefano"). Applicants respectfully traverse this rejection.

The Office Action cites Stefano for teaching the use of betaine in an elution buffer and states that it would have been obvious to one of ordinary skill in the art to modify the combined method of Morrow and Mirzabekov and utilize betaine to elute labeled nucleic acid from a solid support.

Stefano does not rectify the deficiencies of Morrow and Mirzabekov. Stefano fails to teach or suggest chemically fragmenting DNA or RNA in the presence of at least one multivalent metal cation and it fails to teach or suggest attaching a labeling agent at freed terminal phosphates located at the 3' end and/or 5' end.

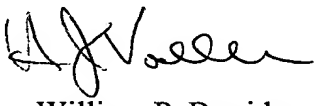
Accordingly, the combination of Morrow, Mirzabekov and Stefano does not disclose, teach or suggest all of the limitations of the claimed invention. Reconsideration and withdrawal of the rejection are respectfully requested.

IV. Conclusion

In view of the above amendments and remarks, Applicants submit that this application is in condition for allowance. Favorable reconsideration and prompt allowance of the application are earnestly solicited.

Should the Examiner believe that anything further would be desirable in order to place this application in better condition for allowance, the Examiner is invited to contact Applicants' undersigned representative at the telephone number listed below.

Respectfully submitted,



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Attachments:
Appendix

WPB:HJV/tea

Date: May 19, 2003 (Corrected Amendment - Original Amendment Timely Filed April 4, 2003)

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DEPOSIT ACCOUNT USE AUTHORIZATION
Please grant any extension necessary for entry;
Charge any fee due to our Deposit Account No. 15-0461

APPENDIX

Changes to Claims:

Claims 38 and 39 are added.

The following is a marked-up version of the amended claims:

1. (Amended) A process ~~of~~ for fragmenting and labeling at least one synthetic or natural member selected from the group consisting of DNA and chimeric DNA-RNA polymers, comprising the steps of:

chemically fragmenting said member in the presence of at least one multivalent metal cation in ~~a substantially~~ an aqueous solution, to produce a plurality of DNA or RNA fragments having freed terminal phosphates for further reaction; and

attaching ~~at least one label to at least one~~ a labeling agent on a plurality of said fragments ~~with a labeling agent to produce a detectably labeled fragment in said aqueous solution~~ at freed terminal phosphates located at the 3' end and/or 5' end of said fragments.

3. (Amended) The process according to claim 1, wherein ~~reagents used in~~ the fragmenting and attaching steps ~~are added to~~ take place in an *in vitro* nucleic acid amplification mixture.

4. (Amended) A process ~~of~~ for fragmenting and labeling a synthetic or natural DNA or RNA nucleic acid, comprising the steps of:

chemically fragmenting ~~the~~ said nucleic acid in the presence of at least one multivalent metal cation in ~~a substantially~~ an aqueous solution, to produce a plurality of DNA or RNA fragments having freed terminal phosphates for further reaction;

attaching ~~at least one label to at least one of~~ a labeling agent on a plurality of said fragments ~~with a labeling agent to produce a detectably labeled fragment in said aqueous solution~~ at freed terminal phosphates located at the 3' end and/or 5' end of said fragments; and ~~then~~

treating said aqueous solution to decrease or eliminate unattached labeling agent.

15. (Amended) The process according to claim 7, wherein the treating step precipitates the labeled nucleic acid fragment at ambient temperature from a solution that contains betaine, ~~DTAB~~ dodecyl trimethylammonium bromide (DTAB) and unlabeled nucleic acid.

25. (Amended) The process according to claim 23, wherein the chemical catalyst is selected from the group consisting of N-methylimidazole, ~~MOPS~~3-(N-morpholino) propane sulfonic acid (MOPS), ~~HEPES~~N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), ~~PIPES~~piperazine-N,N'-bis (2-ethane sulfonic acid) (PIPES), and bioorganic polyamines.

26. (Amended) A process ~~of~~ for fragmenting and labeling a synthetic or natural DNA or RNA nucleic acid, comprising the steps of:

chemically fragmenting ~~the~~ said nucleic acid in the presence of at least one multivalent metal cation selected from the group consisting of Ba^{2+}, Be^{2+}, Cd^{2+}, Ce^{3+}, Cr^{3+}, Eu^{3+}, Fe^{2+}, In^{3+}, Lu^{3+}, Ni^{2+}, Pb^{2+}, Ru^{3+}, Sr^{2+}, Tb^{3+}, Tm^{3+} and Yb^{3+} in ~~a substantially~~ an aqueous solution to produce a plurality of DNA or RNA fragments having freed terminal phosphates for further reaction; and

attaching ~~at least one label to at least one of~~ a labeling agent on a plurality of said fragments ~~with a labeling agent to product a detectably labeled fragment in said aqueous solution~~ at freed terminal phosphates located at the 3' end and/or 5' end of said fragments.